

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

REMARKS

Upon entry of the amendment, claims 201-209 and 212-243 are pending in the application. Claims 202-208, 215-219, 224-237 and 240-243 are withdrawn. Claims 210 and 211 are cancelled. Applicants reserve the right to pursue all cancelled subject matter in a continuing application or applications. Claims 201, 212-214 and 239 are amended. Support for amendments to claim 201 is found throughout the instant specification, as indicated herein. Amendments to claims 212-214 and 239 are clerical in nature. No new matter is added.

Priority

According to the records on file at the U.S. Patent and Trademark Office, the earliest priority date assigned the instant application is January 23, 2003 (corresponding to priority application PCT/IL03/00062 filed on that date). Applicants submit that the instant application is entitled to an earliest priority date of August 19, 2002 (corresponding to priority applications U.S.S.N. 60/404,137 and 60/404,145 filed on that date) as indicated on the Combined Oath/Declaration, Preliminary Amendment and Request for Filing a Continuation Patent Application filed on January 29, 2004.

Due to the error in the earliest priority date, Applicants filed a Request for Corrected Filing Receipt on December 28, 2004.

A Response to the December 28, 2004 Request for Corrected Filing Receipt mailed January 13, 2005 indicated that the applications to which priority is claimed (60/404,137 and 60/404,145) were filed over a year prior to the filing date of the instant application (10/767,064). As such, those referenced applications (60/404,137 and 60/404,145) could not be claimed as domestic or foreign priority.

Applicants disagreed and filed an additional Request for Corrected Filing Receipt on March 6, 2007 describing the relationship of these applications and indicating that, in fact, referenced applications (60/404,137 and 60/404,145) could be claimed as domestic or foreign priority. To date, the U.S. Patent and Trademark Office has not taken any action on the March 6, 2007 Request.

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

Applicants herein submit that the instant application is entitled to the benefit of, and priority to, an earliest priority date of August 19, 2002 (corresponding to priority applications U.S.S.N. 60/404,137 and 60/404,145 filed on that date).

The instant application is a continuation application and claims the benefit of, and priority to, PCT/IL03/00681, filed August 17, 2003, under 35 USC § 365(c). PCT/IL03/00681, filed August 17, 2003, properly claims the benefit of, and priority to, the following applications:

PCT/IL03/00064 filed January 26, 2003;
PCT/IL03/00062 filed January 23, 2003;
Israel Patent Application IL152904 filed November 17, 2002;
U.S. Patent Application 60/404,137 filed August 19, 2002;
U.S. Patent Application 60/452,545 filed March 7, 2003; and
U.S. Patent Application 60/404,145 filed August 19, 2002.

As the instant application is a continuation application of PCT/IL03/00681, filed August 17, 2003, which properly claims the benefit of, and priority to, U.S. Patent Application 60/404,137, filed August 19, 2002 and U.S. Patent Application 60/404,145, filed August 19, 2002, Applicants submit the instant application is entitled to claim priority to these applications.

Correction and recognition of the earliest priority date (August 19, 2002) of the instant application is respectfully requested.

Amendments To The Specification

The Examiner has objected to the inclusion of embedded hyperlinks in the specification, on pages 41 and 45. Hyperlinks have been removed. The reference to Itoh et al. on page 45 has been amended as required.

Withdrawal of the objection is respectfully requested.

35 U.S.C. § 112, First Paragraph, Rejections

Claims 210, 209-214, 238 and 239 are rejected under 35 U.S.C. 112, first paragraph as failing to satisfy the enablement requirement. Claims 210-211 are cancelled, rendering moot the rejections thereof. Claims 201, 212-214 and 239 are

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

amended. Applicants traverse the rejection with respect to the claims as amended herein.

The Examiner, while acknowledging that the specification is enabling for:

A method of expanding an *ex-vivo* population of CD34+ and CD34+/CD38- hematopoietic stem cells in culture while at the same time inhibiting differentiation of said cells *ex-vivo* in a culture medium, the method comprising :

(a) providing hematopoietic mononuclear cells that are not enriched prior to culturing, culturing said mononuclear cells *ex-vivo* in culture under conditions allowing for proliferation and at the same time inhibiting differentiation, said conditions comprising providing either (i) early acting cytokines selected from the group consisting of SCF, FLT3 ligand, IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, TNF- α and thrombopoietin ; and/or (ii) a late acting cytokine selected from the group consisting of GCSF, G/MCSF, and EPO; and

(b) culturing said mononuclear cells in the presence of the copper chelator TEPA ;

thereby expanding the population of said hematopoietic stem cells while inhibiting the differentiation of said hematopoietic stem cells *ex-vivo* in culture;

has alleged that the specification does not provide enablement for expanding any other population of hematopoietic stem cell or culturing mononuclear cells in the presence of any other conditions for proliferation or in the presence of any other copper chelator.

Applicants disagree.

The Examiner has alleged that neither the prior art nor the instant specification has identified a subset of CD34- population of cells capable of providing CD34+ cells. To support this assertion, the Examiner has cited a post-filing article by Hofmeister et al, which allegedly teaches that "Isolating cells...on the basis of ...surface protein expression is likely to include undifferentiated and mature cells...".

Applicants submit that the claimed methods of the instant invention are directed to the expansion and inhibition of differentiation of hematopoietic stem cells by providing copper chelators. Expansion of hematopoietic stem cells and progenitor cells using copper chelators has been demonstrated by the inventors for populations of

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

CD34+ cells, CD34+/CD38- cells, CD133+ cells. The instant specification defines hematopoietic stem cells as those cells including CD34+, CD34+/CD38-, and CD133+ cells (see page 34, lines 5-13 of the instant specification). The specification also teaches methods for identification and isolation of the hematopoietic stem cell populations found in mononuclear cells (see, for example, Examples 1-3 of the instant specification). The expansion of hematopoietic stem cells and progenitor cells using copper chelators has been further demonstrated by the inventors for populations of CD34+ cells, CD34+/CD38- cells, CD133+ cells (see, for example, Peled et al, Cytotherapy 2004;6:344-355, abstract enclosed) and other hematopoietic stem and progenitor populations (see, for example, PCT Application WO03/062404 to Peled, Figures 12a-c, 13a-c, and 14a-b).

Thus, Applicants submit that the instant specification permits one of ordinary skill in the art to make and use the present invention for the expansion of all hematopoietic stem cells, characterized by a variety of markers, from the unselected mononuclear fraction.

Regarding conditions allowing cell proliferation, Applicants submit that instant invention as claimed is directed the expansion of hematopoietic cells from unselected cells by provision of a transition metal chelator in the medium, in the presence of nutrients and cytokines, suitable for the proliferation of hematopoietic cells. It will be appreciated that the feature of the presence of specific combinations of cytokines and nutrients, as for example disclosed in Example 1 is but one embodiment of the actual invention:

"Providing the *ex-vivo* grown cells with conditions for *ex-vivo* cell proliferation include providing the cells with nutrients and preferably with one or more cytokines, as is further detailed hereinunder" (See, page 35, lines 24-26 of the instant specification)

Indeed, as noted by the Examiner, combinations of cytokines and nutrients, in a feeder-layer free culture of stem cells, and without the copper chelator of the claimed invention, are relatively ineffective for expansion of undifferentiated stem and progenitor cell phenotypes:

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

"It is emphasized that considering the level of variation among the mobilized peripheral blood samples, there was no significant difference among such cultures which resulted in maintenance or a small increase of CD34+Thy-1+ cell number" (See, Office Action at page 11)

It will be further appreciated that the instant inventors have demonstrated that the addition of a transition metal chelator having affinity for copper and capable of reducing available intracellular copper effectively inhibits differentiation of stem and progenitor cells cultured in a variety of combinations of cytokines and nutrients (see, for example, US Patent No. 6,962,698, issued Nov. 8, 2005, to Peled et al., Example 1, Table 1, Figures 12-14 and Figure 20). Thus, Applicants submit the instant specification permits one of ordinary skill in the art to make and use a variety of conditions suitable for proliferation of hematopoietic stem cells from an unselected source.

The Examiner has further asserted that one of ordinary skill in the art would be required to engage in undue experimentation in order to determine the role of copper chelators in the expansion of hematopoietic stem cells. Applicants disagree.

As described above, the instant inventors have repeatedly shown that copper chelators capable of reducing intracellular available copper concentration can effectively inhibit differentiation of stem and progenitor cells in *ex-vivo* culture. Good correlation has been found between the ability of chelators to modulate cellular copper content and their biological activities: chelators that reduce cellular copper content are potent differentiation inhibitors. Differentiation inhibitory chelators, such as TEPA, PEHA etc., were found to inhibit differentiation (see, for example, US Patent 6,962,698, Table 3).

The abovementioned notwithstanding, and in order to expedite prosecution in this case, Applicant has chosen to amend claim 201 to include the limitations of "...CD34+, CD34+CD38- and/or CD 133+ hematopoietic stem cells...", "...said conditions comprising providing nutrients and at least an early acting cytokine or cytokines ..." and "...a copper chelator capable of reducing intracellular available copper concentration...". Thus, claim 201 and claims dependent therefrom now read on methods for expansion of defined populations of hematopoietic stem cells from

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

unselected mononuclear cell fraction under defined conditions for proliferation of the cells, by providing a copper chelator capable of reducing intracellular available copper concentration. Support for such amendments is found throughout the instant specification, for example, see page 27, lines 1-5; page 34, lines 5-13 and page 86, lines 25-33.

In view of the foregoing arguments and amendments, Applicants submit that one of ordinary skill in the art would be able to use the claimed methods, as taught, without undue experimentation. Reconsideration and withdrawal of the instant rejections is therefore respectfully requested.

35 U.S.C. § 112, Second Paragraph, Rejections

Claims 210, 209-214, 238 and 239 are rejected under 35 U.S.C. 112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. Claims 210 and 211 are cancelled, rendering moot the rejections thereof. Claims 201, 212-214 and 239 are amended. Applicants traverse the rejection with respect to the claims as amended herein.

The Examiner has rejected claim 201 for reciting "substantially inhibiting differentiation" asserting that the term "substantially" is relative and renders the claim indefinite. Claim 201 is amended herein to recite "inhibiting differentiation".

The Examiner has rejected claim 239 for reciting "hematopoietic". Claim 239 is amended herein to recite "hematopoietic mononuclear cells".

In view of the foregoing amendments, Applicants submit that the pending claims, as amended herein, point out and distinctly claim the subject matter which the Applicant regards as his invention and that one of ordinary skill in the art would readily determine the metes and bounds of the subject matter claimed herein.

Thus, Applicants request reconsideration and withdrawal of the present rejection.

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

35 U.S.C. § 102 Rejection

Claims 201, 209-214 and 238-239 are rejected under 35 U.S.C. §102(a) as being anticipated by Peled et al (Blood, November 16 2002;100, No 11, Abstract 4076) ("Peled"). The Examiner states that Peled discloses all the steps recited in the claims.

As described under the **Priority** section *supra*, Applicants submit that the instant specification is entitled to the benefit of, and priority to, an earliest priority date of August 19, 2002 (corresponding to priority applications U.S.S.N. 60/404,137 and 60/404,145 filed on that date).

Applicants understand that Peled was made available to the public on November 16 2002. As such, Applicants submit that the Peled reference does not qualify as prior art under 35 U.S.C. §102(a) as the Peled publication does not describe an invention that was known or used by others before invention by Applicants under 35 U.S.C. § 102(a).

Accordingly, Peled is not prior art to the instant claims. The rejection should be withdrawn.

35 U.S.C. § 103 Rejection

Claims 201, 209-214 and 238-239 are rejected under 35 U.S.C. §103(a) as being unpatentable over Sandstrom et al (Blood, 1995;86(3):958-70) and Peled. As described above, Peled does not qualify as prior art to the instant invention; and as such, this rejection should be withdrawn.

APPLICANTS: Peled et al.
U.S.S.N.: 10/767,064

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



Ivor R. Elrifi, Reg. No. 39,529
Matthew Pavao, Reg. No. 50,572
Attorneys for Applicants
c/o MINTZ LEVIN
Tel.: (617) 542-6000
Fax: (617) 542-2241
Customer No.: 30623

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